

Sex as a Factor in Conversion From Laparoscopic Cholecystectomy to Open Surgery

Serdar Yol, Adil Kartal, Celalettin Vatansev, Faruk Aksoy, Hatice Toy, MD

ABSTRACT

Objectives: Although laparoscopic cholecystectomy has become the standard treatment for symptomatic gallbladder diseases, conversion to open surgery is required in a substantial proportion of patients. In this study, we attempted to clarify whether male sex carries an increased risk for conversion to open surgery during laparoscopic cholecystectomy.

Methods: This study comprised 80 patients (41 females, 39 males) with symptomatic gallbladder stones. Average age was 39.2 years, and all female patients were of reproductive age. Patients were excluded from the study if they had acute cholecystitis, previous abdominal surgery, systemic or connective tissue diseases, or were using tobacco, alcohol, or medications that affect wound healing or inflammation. Tissue samples were obtained from the same sites in each gallbladder wall and pericholecystic tissue for the measurement of tissue hydroxyproline (HP) and collagen. Samples were examined under light microscopy for histopathology. Findings in male and female patients were compared by using the Student *t* test.

Results: All patients except 3 males received laparoscopic cholecystectomy. Conversion to open cholecystectomy was necessary in those 3 because of intense pericholecystic fibrosis. In male patient samples, macrophages were twice as numerous as in female samples, whereas mast cells in the men were 4 times more numerous, and eosinophils were 6 times more numerous ($P < 0.01$). In men, HP levels in the gallbladder wall and pericholecystic tissue were $23.4 \pm 14.9 \mu\text{g}/\text{mg}$ dry tissue and $25.2 \pm 13.1 \mu\text{g}/\text{mg}$ dry tissue, respectively. The corresponding values in women were $13.1 \pm 9.4 \mu\text{g}/\text{mg}$ dry tissue and $14.5 \pm 8.1 \mu\text{g}/\text{mg}$ dry tissue.

This higher level of tissue HP in men was statistically significant ($P < 0.015$). Tissue collagen levels both in the submucosal area of the gallbladder wall and in pericholecystic tissue were significantly higher in men than in women ($P < 0.05$).

Conclusion: Our data suggest that in the context of symptomatic gallbladder stones, inflammation and fibrosis are more extensive in men than in women. These findings may help explain why the rate of conversion to open surgery is higher in men than in women.

Key Words: Cholecystectomy, Sex, Conversion to open surgery, Tissue hydroxyproline level, Tissue collagen level.

INTRODUCTION

For the treatment of symptomatic calculous cholecystitis, laparoscopic cholecystectomy has become standard. However, not every cholecystectomy that begins laparoscopically can be completed that way. Conversion to open surgery has been found to occur at rates of 3% to 10%.¹ Risk factors for conversion include massive fibrosis, anatomic anomalies, acute cholecystitis, intraoperative complications (bleeding, internal organ trauma), old age, sex, history of previous upper abdominal surgery, and lack of appropriate laparoscopy instruments.²⁻⁴ We believe that among these, sex has not been investigated sufficiently. According to our experience and the research literature,⁵ laparoscopy is more difficult and time consuming in men than in women. The greater difficulty we have encountered in men is related to the presence of fibrosis.

In 80 patients with calculous gallbladders, we measured collagen and hydroxyproline (HP) in the gallbladder wall and in nearby adhesions. Inflammatory cells in the gallbladder wall were also evaluated. In this way, an attempt was made to analyze the severity of inflammation and fibroplasia in the gallbladder and nearby tissue, and these were compared in male versus female patients.

Department of General Surgery, Selcuk University, Meram Medical Faculty, Akyokus, Konya, Turkey (Professors Yol, Kartal, Vatansev, Aksoy).

Department of Pathology, Selcuk University, Meram Medical Faculty, Akyokus, Konya, Turkey (Dr Toy).

We thank Greg Hammond at the Louisiana Society of Anesthesiologists for his kind proofreading of the manuscript.

Address reprint requests to: Serdar Yol, Associate Professor, Necip Fazil Mah. Evliya Celebi Cad., Karakoc Apt. 31/5, Yaka-Meram, Konya, TURKEY. Telephone: +90 533 420 1114, Fax: +90 332 223 6184, E-mail: serdaryol@hotmail.com

© 2006 by JSLs, Journal of the Society of Laparoendoscopic Surgeons. Published by the Society of Laparoendoscopic Surgeons, Inc.

METHODS

The study included 80 patients with symptomatic chronic calculous cholecystitis (41 women, 39 men). Average age was 39.2 (range, 25 to 43). All the women were of child-bearing age. Patients were excluded from the study if they had any of the following: acute cholecystitis, gallstone pancreatitis, biliary colic, systemic or connective tissue disease, abnormal laboratory values, previous abdominal surgery, alcohol or cigarette use, oral contraceptive use, or use of medications that affect wound healing or inflammation (eg, steroids, anti-inflammatory agents).

Fibrotic tissue taken from the gallbladder's immediate surroundings was divided into 2 portions. One portion was frozen in physiologic saline at -60°C for hydroxyproline (HP) analysis. The other portion was sent to the laboratory for collagen analysis. The gallbladder was incised through its long axis. The gallbladder's interior was washed out after its contents were emptied. In each patient, 2 full-thickness specimens 1cm in diameter were excised from Hartman's pouch. One was used for HP analysis, and the other was evaluated for collagen and inflammatory cells. HP was analyzed by means of a modified Woessner method, as $\mu\text{g}/\text{mg}$ dry tissue.⁶

For cellular analysis, wall samples were kept in formalin-sucrose solution (pH 6.8) for 21 hours at 4°C and then in Holt solution for another 21 hours. After routine procedures were followed, 12-micron sections were stained with Pappenheim's panoptic stain. Under this stain, lymphocytes were seen as cells containing red-brown dots, and macrophages were seen as cells containing widespread granules. Mast cells and eosinophils were also seen. For all cells, these observations were made with a light microscope (Laborlux 12, Leitz, Germany) in 10 random fields (UA: $9 \times 10^4 \mu\text{m}^2$) at 40-power magnification, and percentages of cell types were documented.

Collagen was evaluated histologically in the samples of gallbladder adhesion tissue. Samples were fixed in neutral formalin and were then embedded in paraffin blocks, from which 5-micron sections were taken. After being stained with Masson trichrome histochemical stain, the sections were examined by light microscopy and collagen fibers under the tunica mucosa were evaluated. In this stain, nucleus was dark red, muscle was red, and collagen tissue was light green.

All results were analyzed as a male versus female comparison via the Student *t* test. For conversion rates between 2 sexes, the Fisher exact test was used.

RESULTS

In 3 of the male patients, laparoscopic cholecystectomy was converted to open surgery due to massive fibrosis. All other patients underwent laparoscopic cholecystectomy only. The difference was not significant statistically ($P=0.111$). Routine histopathological examination of resected gallbladders was reported as chronic calculous cholecystitis in all patients.

Inflammatory cells were more numerous in the tissue samples taken from men. Macrophages were twice as numerous in males compared with macrophages in females. Mast cells were 4 times more numerous, and eosinophils were 6 to 7 times more numerous in men. All of these differences were statistically significant ($P<0.01$). No difference existed between the sexes in terms of lymphocyte count (**Figure 1**).

HP values in men were $23.4 \pm 14.9 \mu\text{g HP}/\text{mg}$ dry tissue in the gallbladder wall and $25.2 \pm 13.1 \mu\text{g HP}/\text{mg}$ dry tissue in pericholecystic adhesions. In women, these values were $13.1 \pm 9.4 \mu\text{g HP}/\text{mg}$ dry tissue in gallbladder wall and $14.5 \pm 8.1 \mu\text{g HP}/\text{mg}$ dry tissue in adhesions. The difference in HP values between male and female patients was found to be significant ($P<0.015$).

Dense collagen accumulation was seen in the submucosal region of the gallbladder wall in males, whereas collagen was notable in the gallbladder wall in only 6 of the female patients, and then only mildly so (**Figures 2 and 3**). In females, collagen accumulation in nearby tissues was slightly more evident than that in the gallbladder wall.

DISCUSSION

Laparoscopic cholecystectomy has become a readily available procedure for extracting the gallbladder in indicated patients. In some patients, however, the need arises during this procedure to convert to open surgery. A range of risk factors has been proposed for this conversion.^{1,2,4,7-11}

The sex of the patient has been discussed as a risk factor for conversion to open surgery, with some authors questioning its relevance.^{7,10,11} One study that found a higher conversion rate among males suggested that gallbladder disease in males is a different entity.¹² The same study argued that males pay less attention to their health problems and permit them to advance, and by the time these patients seek treatment the stage has been set for a difficult laparoscopic cholecystectomy. Another study that found a higher conversion rate among males attributed it to a greater incidence of gallbladder and biliary tree ana-

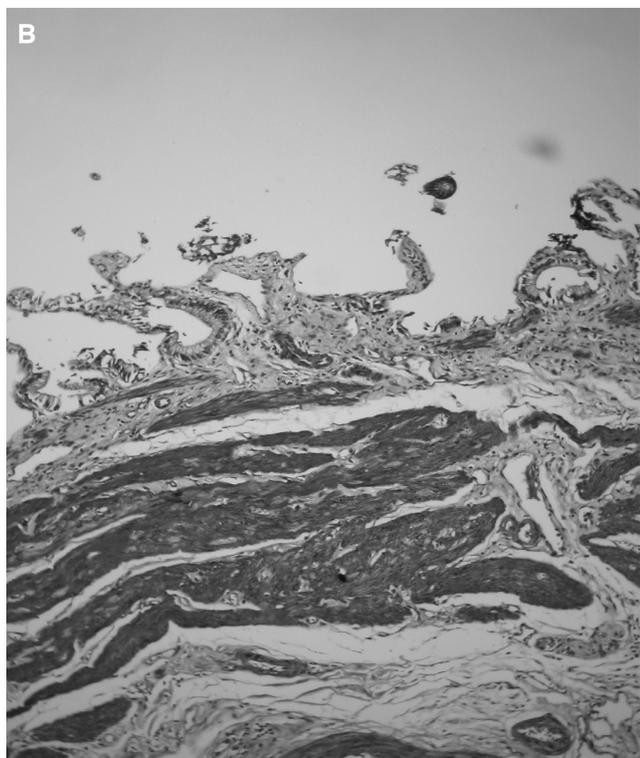
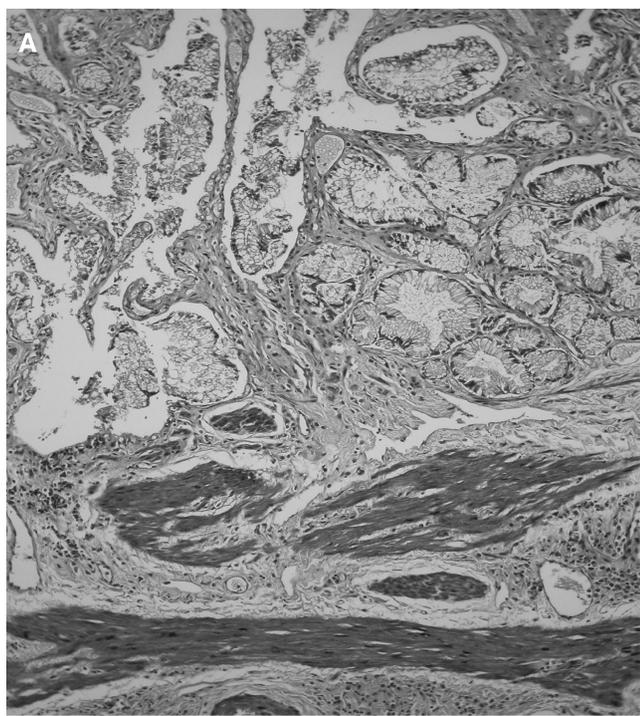


Figure 1. Massive inflammatory cells in the gallbladder wall of a male patient (A); Comparatively fewer inflammatory cells in a female patient (B).

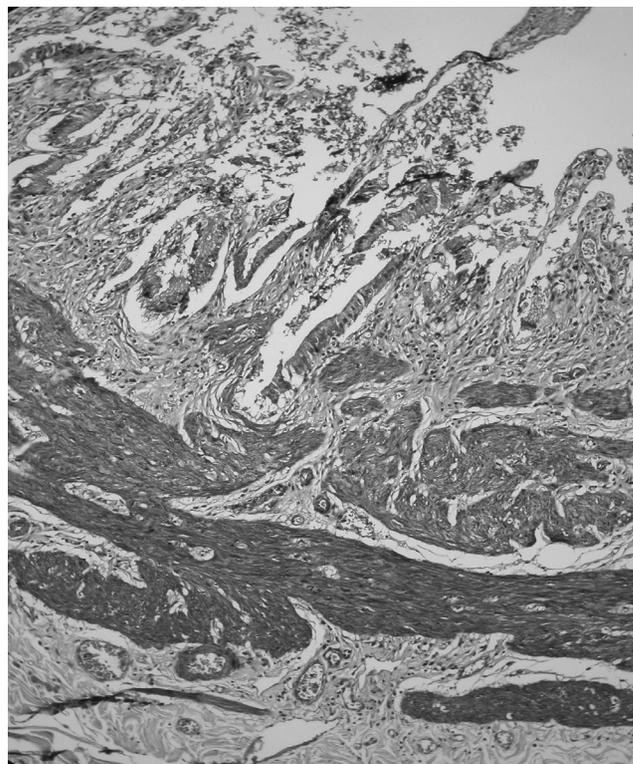


Figure 2. Massive submucosal collagen accumulation in a male patient.

tomic difficulties in males.⁴ Very few data are available, however, in terms of the biochemical phenomena, such as inflammation that might be involved in the higher rates of conversion to open surgery in males.

An inflammatory process can end with little collagen accumulation, and it can even heal without a scar.⁹ However, severe acute cholecystitis and chronic cholecystitis often involve massive collagen production and fibrosis. The collagen is produced by fibroblasts during inflammation and wound healing. The main cells involved in the inflammatory phase are macrophages, while during the proliferation phase fibroblasts are more predominant. The variability of these processes raises the question of whether they might be related to the particular organ involved, such as the gallbladder, or to biochemical characteristics of patients, such as sex hormones.

Shamberger et al¹³ investigated the effects of testosterone on the wounds of normal and castrated rats. In castrated rats, they found a 15% decrease in shearing resistance at 14 days but no difference at 21 days. After giving exogenous testosterone, they found no effect (positive or negative) on wound healing, ie, on collagen accumulation in

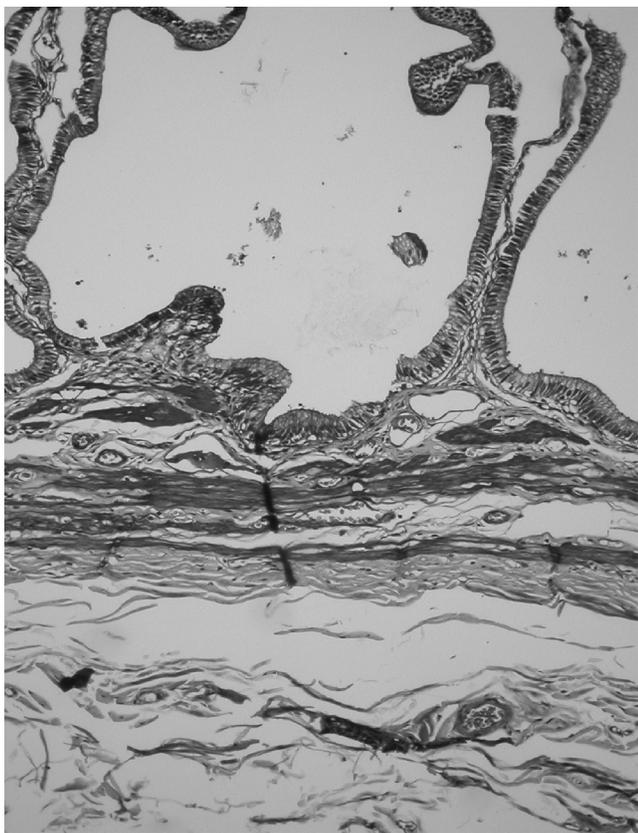


Figure 3. Virtual absence of collagen accumulation in a female.

normal and castrated rats. Few studies have investigated effects of testosterone on wound healing, but a good number of studies have examined the effects of testosterone on trauma. Whichman et al¹⁴ found that after traumatization and bleeding of castrated rats, a significant decrease occurred in synthesis of IL-1 and IL-6 by macrophages. Angele et al¹⁵ induced hemorrhagic shock in male rats that had been given the testosterone antagonist flutamide, resuscitated them, and then introduced sepsis. They found that immune suppression was prevented, and that death due to sepsis was decreased. This was corroborated by a prospective study in humans diagnosed with sepsis,¹⁶ which found a relation between sex hormone levels, inflammatory mediators, and a better prognosis in women.

Connective tissue and autoimmune diseases like scleroderma, lupus erythematosus,¹⁷ and rheumatoid arthritis are more prevalent in females of reproductive age than in postmenopausal females or in men. These diseases decrease in severity when estrogen levels are high.^{5,18–21} It is also interesting that estrogen receptors are expressed by macrophages, monocytes, lymphocytes, and mast cells,

and it is accepted that estrogen directly affects their function and suppresses their cytokine production.^{8,9,18,22,23} Estrogen replacement has been shown to inhibit peritoneal adhesions in ovariectomized mice, and appears to inhibit macrophage accumulation as well.²⁰ Macrophages in mice produce fewer cytokines (IL-1, IL-6, and TNF alpha) when exposed to 17-beta estradiol,²⁴ and estrogen also appears to regulate mRNA expression in fibroblasts.²²

The links between sex hormones and inflammation suggested by the studies above are consistent with the findings of a retrospective study by Kanaan et al,⁸ which found severe inflammation and male sex to be risk factors for conversion from laparoscopic to open cholecystectomy. In that study, the pericholecystic tissue of inflamed gallbladder showed more severe fibrosis in male patients, and this was associated with difficult dissection and a higher rate of conversion to open surgery.

The higher levels of collagen and HP found in the samples from male patients in this study suggest a difference in the cellular processes of inflammation. The higher numbers of macrophages, mast cells, and eosinophils found in the samples from male patients corroborate this, and show the difficulty of trying to pinpoint causality in something as complex as the inflammation cascade. The increased number of macrophages in men indicates the severity of inflammation and high levels of cytokines. The cytokines mediate interactions between macrophages and fibroblasts in the next phase of the inflammatory process. More active fibroblasts mean more collagen and fibrosis. Mast cells release histamine and chemotactic factors for neutrophils and eosinophils, thereby expanding the cascade of cell types involved in inflammation.

Although the rate of conversion to open cholecystectomy was clearly higher in men than in women of reproductive age, the difference was not statistically significant probably due to the sample size. Also it is unknown whether such a difference may exist between postmenopausal women and men of comparable age. This is an interesting question for further study. If inflammation and fibrosis in gallbladder patients are related to the biochemical aspects of being male or female, then less of a difference would be expected. However, aging alone is a risk factor for conversion to open surgery, and if we add other systemic diseases and medications, a study capable of addressing this question would be difficult to perform. The present study is part of an initiative in our department toward addressing these broader questions.

CONCLUSION

The results of this study suggest that in men with symptomatic gallbladder stones, inflammation and fibrosis occur more than they do in women with the same disease. This translates into difficult dissections during laparoscopic cholecystectomy and a higher rate of conversion to open surgery in male patients.

References:

- Sanabria JR, Gallinger S, Croxford R, Strasberg SM. Risk factors in elective laparoscopic cholecystectomy for conversion to open cholecystectomy. *J Am Coll Surg*. 1994;179:696–704.
- Lo CM, Fan ST, Liu CL, Lai EC, Wong J. Early decision for conversion of laparoscopic to open cholecystectomy for treatment of acute cholecystitis. *Am J Surg*. 1997;173:513–517.
- Vatansev C, Kartal A, Calayan O, Vatansev H, Yol S, Tekin A. Why is the conversion rate to open surgery during cholecystectomy higher in men than in women? Proceedings of the Turkish National Surgery Congress, Turkey 2002;p. 177.
- Zisman A, Gold-Deutch R, Zisman E, Negri M, Halpern Z, Lin G, Halevy A. Is male gender a risk factor for conversion of laparoscopic into open cholecystectomy? *Surg Endosc*. 1996;10:892–894.
- Gharaibeh KI, Qasaimeh GR, Al-Heiss H, et al. Effect of timing of surgery, type of inflammation, and sex on outcome of laparoscopic cholecystectomy for acute cholecystitis. *J Laparoendosc Adv Surg Tech A*. 2002;12:193–198.
- Woessner JF Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. *Arch Biochem Biophys*. 1961;93:440–447.
- Alponat A, Kum CK, Koh BC, Rajnakova A, Goh PMY, Mouiel J. Predictive factors for conversions of laparoscopic cholecystectomy. *World J Surg*. 1997;21(6):629–633.
- Kanaan SA, Murayama KM, et al. Risk factors for conversion of laparoscopic to open cholecystectomy. *J Surg Res*. 2002;106:20–24.
- Kartal A, Aksoy F, Vatansev C, et al. Does estrogen cause low conversion rates in laparoscopic cholecystectomies for acute and chronic cholecystitis in women? *JLSL*. 2001;5:309–12.
- Liu CL, Fan ST, Lai EC, Lo CM, Chu KM. Factors affecting conversion of laparoscopic cholecystectomy to open surgery. *Arch Surg*. 1996;131:98–101.
- Wiebke EA, Pruitt AL, Howard TJ, et al. Conversion of laparoscopic to open cholecystectomy. An analysis of risk factors. *Surg Endosc*. 1996;10:742–745.
- Russell JC, Walsh SJ, Reed-Fourquet L, Mattie A, Lynch J. Symptomatic cholelithiasis: a different disease in men? Connective Tissue Laparoscopic Cholecystectomy Registry. *Ann Surg*. 1998;227:195–200.
- Shamberger RC, Thistlethwaite PA, Thibault LE, Talbot TL, Brennan MF. The effect of testosterone propionate on wound healing in normal and castrate rats. *J Surg Res*. 1982;33:58–68.
- Wichmann MW, Ayala A, Chaudry IH. Male sex steroids are responsible for depressing macrophage immune function after trauma-hemorrhage. *Am J Physiol*. 1997;273:C1335–C1340.
- Angele MK, Wichmann MW, Ayala A, Cioffi WG, Chaudry IH. Testosterone receptor blockade after hemorrhage in males. *Arch Surg*. 1997;132:1207–1214.
- Schroder J, Kahlke V, Staubach KH, Zabel P, Stuber F. Gender differences in human sepsis. *Arch Surg*. 1998;133:1200–1205.
- Inman RD. Immunologic sex differences and the female predominance in systemic lupus erythematosus. *Arthritis Rheum*. 1978;21:849–852.
- Frazier-Jessen MR, Kovacs EJ. Abdominal wall thickness as a means of assessing peritoneal fibrosis in mice. *J Immunol Methods*. 1993;162:115–121.
- Frazier-Jessen MR, Kovacs EJ. Estrogen modulation of JE/monocyte chemoattractant protein-1 mRNA expression in murine macrophages. *J Immunol*. 1995;154:1838–1845.
- Frazier-Jessen MR, Mott FJ, Witte PL, Kovacs EJ. Estrogen suppression of connective tissue deposition in a murine model of peritoneal adhesion formation. *J Immunol*. 1996;156(8):3036–3042.
- Hudson I, Hopwood D. Macrophages and mast cells in chronic cholecystitis and “normal” gallbladders. *J Clin Pathol*. 1986;39:1082–1087.
- Kovacs EJ, Faunce DE, Ramer-Quinn DS, Mott FJ, Dy PW, Frazier-Jessen MR. Estrogen regulation of JE/MCP-1 mRNA expression in fibroblasts. *J Leukoc Biol*. 1996;59:562–568.
- Weusten JJ, Blankenstein MA, Gmelig-Meyling FH, Schuurman HJ, Kater L, Thijssen JH. Presence of oestrogen receptors in human blood mononuclear cells and thymocytes. *Acta Endocrinol (Copenh)*. 1986;112:409–414.
- Deshpande R, Khalili H, Pergolizzi RG, Michael SD, Chang MD. Estradiol down-regulates LPS-induced cytokine production and NFκB activation in murine macrophages. *Am J Reprod Immunol*. 1997;38:46–54.